CLASP Method — Residual Solvents by GC/FID

1.0 Scope and Application

1.1 This method was adapted from the EPA Method 8015D Non-Halogenated Organics Using GC/FID.

1.2 This method is used to determine the concentrations of residual solvents in a variety of cannabis matrices by gas chromatography flame ionization detector headspace analysis. The following solvents and decision point cutoffs have been established in WAC 314-55-102.

Solvent	µg/g	ppm (simplified)	CAS #
Acetone	$5.0 * 10^3$	5000	67-64-1
Benzene	2	2	71-43-2
Butanes (Sum of Isomers)	$5.0 * 10^3$	5000	
• n-butane			106-97-8
• 2-methylpropane (isobutane)			75-28-5
Cyclohexane	$3.9 * 10^3$	3880	110-82-7
Chloroform	2	2	67-66-3
Dichloromethane	$6.0 * 10^2$	600	75-09-2
Ethanol	$5.0 * 10^3$	5000	64-17-5
Ethyl acetate	$5.0 * 10^3$	5000	141-78-6
Heptanes (Single Isomer)	$5.0 * 10^3$	5000	
• n-heptane			142-82-5
Hexanes (Sum of Isomers)	$2.9 * 10^2$	290	
• n-hexane			110-54-3
• 2-methylpentane			107-83-5
• 3-methylpentane			96-14-0
• 2,2-dimethylbutane			75-83-2
• 2,3-dimethylbutane			79-29-8
Isopropanol (2-propanol)	$5.0 * 10^3$	5000	67-63-0
Methanol	$3.0 * 10^3$	3000	67-56-1
Pentanes (Sum of Isomers)	$5.0 * 10^3$	5000	
• n-pentane			109-66-0
• methylbutane (isopentane)			78-78-4
• dimethylpropane (neopentane)			463-82-1
Propane	$5.0 * 10^3$	5000	74-98-6
Toluene	$8.9 * 10^2$	890	108-88-3
Xylenes (Sum of Isomers)	$2.2 * 10^3$	2170	
• 1,2-dimethylbenzene (ortho-)			95-47-6
• 1,3-dimethylbenzene (meta-)			108-38-3
• 1,4-dimethylbenzene (para-)			106-42-3

1.3 This method is applicable to the analysis of other analytes should additional volatiles be added in the future.

1.4 This method is restricted for use by, or under the supervision of, analysts experienced in the use of a gas chromatograph and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 Summary of the Method

2.1 This method provides gas chromatographic conditions for the detection of certain volatile and semi-volatile compounds.

2.2 An appropriate column and temperature program are used in the gas chromatograph to separate the organic compounds. Detection is achieved by a flame ionization detector (FID).

2.3 This method allows the use of packed or capillary columns for the analysis and confirmation of the non-halogenated individual analytes. The GC columns and conditions listed have been demonstrated to provide separation of those target analytes. Other columns and conditions may be employed, provided that the analyst demonstrates adequate performance for the intended application.

3.0 Definitions

Refer to the EPA SW-846 chapter of terms and acronyms for potentially applicable definitions.

4.0 Interferences

4.1 Solvents reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary.

4.2 When analyzing for volatile organics, samples can be contaminated by diffusion of volatile organics (particularly chlorofluorocarbons and methylene chloride) through the sample container septum during shipment and storage. A trip blank prepared from organic-free reagent water and carried through sampling and subsequent storage and handling will serve as a check on such contamination.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device must be rinsed out between samples with an appropriate solvent. Whenever an unusually concentrated sample is encountered, it should be followed by injection of a solvent blank to check for cross contamination.

4.3.1 Clean purging vessels with a detergent solution, rinse with distilled water, and then dry in a 105°C oven between analyses. Clean syringes or autosamplers by flushing all surfaces that contact samples using appropriate solvents.

4.3.2 All glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware and dry it in an oven at 130°C for several hours. Store dry glassware in a clean environment.

4.4 The flame ionization detector (FID) is a non-selective detector. There is a potential for many non-target compounds present in samples to interfere with this analysis. There is also the potential for analytes to be resolved poorly, especially in samples that contain many analytes.

5.0 Safety

There are no significant safety issues specific to this method. However, CLASP standardized methods do not purport to address all safety issues associated with their use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of Occupational Safety and Health Administration OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

The mention of trade names or commercial products in this manual is for illustrative purposes only and does not constitute a CLASP endorsement or exclusive recommendation for use. The products and instrument settings cited in CLASP supported methods represent those products and settings used during EPA method development. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.1 Gas chromatograph: Analytical system complete with gas chromatograph suitable for solvent injections, direct aqueous injection, headspace, and equipped with all necessary accessories, including detectors, column supplies, recorder, gases, and syringes. A software data system for measuring peak heights and/or peak areas is recommended.

6.2 Recommended GC columns

The choice of GC column will depend on the analytes of interest, the expected concentrations, and the intended use of the results. The packed columns listed below are generally used for screening analyses. The capillary columns are recommended for volatile solvents used in the cannabis industry. Other columns and columns of other diameters may be employed if the analyst can demonstrate acceptable performance for the intended application.

6.2.1 Column 1: 8-ft x 0.1-in. ID stainless steel or glass column, packed with 1% SP-1000 on Carbopak-B 60/80 mesh or equivalent.

6.2.2 Column 2: 6-ft x 0.1-in. ID stainless steel or glass column, packed with n-octane on Porasil-C 100/120 mesh (Durapak) or equivalent.

6.2.3 Column 3: 30-m x 0.53-mm ID fused-silica capillary column bonded with DB-Wax (or equivalent), 1-µm film thickness.

6.2.4 Column 4: 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 5% methyl silicone (DB-5, SPB-5, RTx, or equivalent), 1.5-µm film thickness.

6.2.5 Wide-bore columns should be installed in 1/4-inch injectors, with deactivated liners designed specifically for use with these columns.

6.3 Detector: Flame ionization (FID)

6.4 Sample introduction and preparation apparatus

6.4.1 Refer to the headspace sample preparation method for the appropriate apparatus for headspace analyses.

6.4.2 Samples may also be introduced into the GC via injection of solvent extracts or direct injection of aqueous samples.

6.5 Syringes

6.5.1 5-mL Luer-Lok glass hypodermic and 5-mL gas-tight syringe with shutoff valve, for volatile analytes.

6.5.2 Microsyringes: 10- and 25-µL with a 0.006-in. ID needle (Hamilton 702N or equivalent) and 100-µL.

6.6 Volumetric flasks, Class A: Appropriate sizes with ground-glass stoppers.

6.7 Analytical balance: 160-g capacity, capable of measuring to 0.0001 g.

7.0 Reagents and Standards

7.1 Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic free reagent water. All references to water in this method refer to organic-free reagent water.

7.3 Stock standards: Stock solutions may be prepared from pure standard materials or purchased as certified solutions. Since methanol is a target analyte any standards should not be prepared in methanol. Standards must be replaced if comparison with check standards indicates a problem.

7.4 Secondary dilution standards: Using stock standard solutions, prepare secondary dilution standards, as needed, that contain the compounds of interest, either singly or mixed

together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards will bracket the working range of the analytical system. Secondary dilution standards should be stored with minimal headspace for volatiles and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.5 Calibration standards: Calibration standards at a minimum of five different concentrations. For headspace, the standards are prepared as directed in 7.6 below. One of the standards should be at or below the cutoff or decision point of the assay. The remaining concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Each standard should contain each analyte to be determined by this method.

7.6 Calibration spiking solutions: Prepare five or more spiking solutions in water that contain all the target analytes. The concentrations of the calibration solutions should be such that the addition of 1.0 µL of each to the headspace vials will bracket the analytical range of the detector. Alternatively, calibration standards may be prepared by adding different volumes of one or more stock solutions provided that the linearity of the calibration is not affected by the methanol content of the original standard.

7.7 Internal standards (if internal standard calibration is used): To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences.

7.8 Surrogate standards: Whenever possible, the analyst should monitor both the performance of the analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and blank with one or two surrogate compounds which are not affected by method interferences.

8.0 Sample Collection, Preservation, and Storage

8.1 Place measured sample directly into an empty or prepared headspace vials for analysis. If high concentrations of volatiles are expected, decrease the amount in half and multiply the result by two. If results are still not within the linear range for the analyte, decrease the amount again using one quarter of the amount required and multiply the results by four.

8.2 Open only one vial at a time to minimize loss of residual solvents.

9.0 Quality Control

9.1 Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.3 Any method blanks, matrix spike samples, or replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.4 Refer to16-309 WAC for specific quality control (QC) requirements. Quality control must ensure the proper operation of the various sample preparation and/or sample introduction techniques. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

9.5 Quality control procedures necessary to evaluate the GC system operation must include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

9.6 Initial demonstration of proficiency: Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made.

9.7 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

9.7.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

9.7.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

9.8 Surrogate recoveries: The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory.

9.9 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 Calibration and Standardization

See Sec. 11.0 for information on calibration and standardization.

11.0 Procedure

11.1 All internal standards, surrogates, and matrix spikes (when applicable) must be added to samples before introduction into the GC/FID system. Consult the applicable sample introduction method regarding when to add standards. Other sample introduction techniques may be appropriate for specific applications and the techniques described here also may be appropriate for other matrices and analytes. Whatever technique is employed, including those specifically listed below, the analyst must demonstrate adequate performance for the analytes of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Sec. 9.6, using a clean reference matrix.

11.1.1 Automated static headspace: This technique employs a device that collects the volatile organics from the headspace over a sample contained in a sealed vial and introduces them into the GC/FID system.

11.1.2 Solvent injection: This technique involves the syringe injection of solvent extracts of aqueous samples.

11.2 Suggested chromatographic conditions: Establish the GC operating conditions appropriate for the GC column being utilized and the target analytes specified in section 1.1. Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Suggested operating conditions are given below for the columns recommended in Sec. 6.2. The columns listed in this section were the columns used to develop the method performance data and it is not CLASP's intent to exclude the use of other columns. Laboratories may use these columns or other columns provided that they document method performance data (e.g., chromatographic resolution and sensitivity) that meet the data quality needs of the intended application.

11.2.1 Column 1

Carrier gas (helium) flow rate:	40 mL/min
Temperature program	
Initial temperature:	45 °C, hold for 3 minutes
Program:	45 °C to 220 °C, at 8 °C/min
Final temperature:	220 °C, hold for 15 minutes.

11.2.2 Column 2

Carrier gas (helium) flow rate:	40 mL/min
Temperature program	
Initial temperature:	50 °C, hold for 3 minutes
Program:	50 °C to 170 °C, at 6 °C/min
Final temperature:	170 °C, hold for 4 minutes.

11.2.3 Column 3

Carrier gas (helium) flow rate:	15 mL/min
Temperature program	
Initial temperature:	45 °C, hold for 4 minutes
Program:	45 °C to 220 °C, at 12 °C/min
Final temperature:	220 °C, hold for 3 minutes.

11.3 Initial calibration

11.3.1 Set up the sample introduction system as outlined in the method of choice (see Sec. 11.1). A separate calibration is necessary for each sample introduction mode because of the differences in conditions and equipment. Establish chromatographic operating parameters that provide instrument performance appropriate for the intended application. Prepare calibration standards using the procedures described above (see Sec. 7.5).

11.3.2 External standard calibration procedure for single-component analytes.

11.3.2.1 For each analyte and surrogate, prepare calibration standards at a minimum of five different concentrations. For headspace analysis, the standards must be prepared in organic-free reagent water and then spiked into the organic-free water in headspace vial. The spiking solutions must be at concentrations which will dilute to the desired standard concentrations when added into the organic-free water in the headspace vials. Otherwise, standards should be made by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with water or an appropriate solvent not on the list. One of the standards should be at a concentration at or below the quantitation limit necessary for the method (based on the concentration in the final volume described in the preparation method, with no dilutions). The concentrations of the other standards should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

11.3.2.2 Introduce each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Tabulate peak height or area responses against the mass injected. Calculate the calibration factor (CF) for each single-component analyte. Most instrument software will do this for you.

11.3.3 Calibration linearity: The linearity of the calibration must be assessed for every analyte tested.

11.3.3.1 If the percent relative standard deviation (%RSD) of the calibration factors is less than 20% over the working range, then linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

11.3.3.2 If the % RSD is more than 20% over the working range, linearity through the origin cannot be assumed.

11.4 Retention time windows: Single-component target analytes (see Sec. 1.1) are identified on the basis of retention time windows.

11.4.1 Before establishing retention time windows, make sure that the chromatographic system is functioning reliably and that the operating parameters have been optimized for the target analytes and surrogates in the sample matrix to be analyzed.

11.5 Calibration verification

11.5.1 The initial calibration and retention times must be verified at the beginning of each 12-hour work shift, at a minimum. When individual target analytes are being analyzed, verification is accomplished by the analysis of one or more calibration standards (normally mid-concentration, but a concentration at or near the action level may be more appropriate) that contain all of the target analytes and surrogates.

11.5.2 Calculate the % difference. If the response for any analyte is within ±20% of the response obtained during the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the mean CF or RF values from the initial calibration to quantitate sample results. If the response for any analyte varies from the predicted response by more than ±20%, corrective action must be taken to restore the system, or a new calibration curve must be prepared for that compound.

11.5.3 All target analytes and surrogates in the calibration verification analyses must fall within previously established retention time windows. If the retention time of any analyte does not fall within the established window, then corrective action must be taken to restore the system, or a new calibration curve must be prepared for that compound.

11.5.4 Solvent blanks and any method blanks should be run with calibration verification analyses to confirm that laboratory contamination does not cause false positive results.

11.6 Gas chromatographic analysis

11.6.1 Samples are analyzed in a set referred to as an analytical sequence. The sequence begins with calibration verification followed by sample extract analyses. Additional analyses of the verification standard(s) throughout a 12-hour shift are strongly recommended. A verification standard is also necessary at the end of a set (unless internal standard calibration is used). The sequence ends when the set of samples has been injected or when retention time and/or % difference QC criteria are exceeded.

If the criteria are exceeded, inspect the gas chromatographic system to determine the cause and perform whatever maintenance is necessary before recalibrating and proceeding with sample analysis. All sample analyses performed using external standard calibration must be bracketed with acceptable data quality analyses (e.g., calibration and retention time criteria). Therefore, all samples that fall between the standard that failed to meet the acceptance criteria and the preceding standard that met the acceptance criteria must be reanalyzed.

11.6.2 Samples are analyzed with the same instrument configuration as is used during calibration. Analysts are cautioned that opening a sample vial or drawing an aliquot from a sealed vial (thus creating headspace) will compromise samples analyzed for volatiles.

11.6.3 Sample concentrations are calculated by comparing the sample response with the response from the initial calibration of the system (see Sec. 11.3). Therefore, if the sample response exceeds the limits of the initial calibration range, a dilution of the sample or sample extract must be analyzed. Samples and/or sample extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, is acceptable as long as calibration limits are not exceeded. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

11.6.4 The performance of the entire analytical system should be checked every 12 hours, using data gathered from analyses of blanks, standards, and samples. Significant peak tailing must be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, the detector operation, or leaks in the system. Follow manufacturer's instructions for maintenance of the introduction device.

11.7 Instrument Maintenance

11.7.1 Injection of sample extracts often leaves a high boiling residue in the injection port area, splitters (when used), and the injection port end of the chromatographic column. This residue affects chromatography in many ways (i.e., peak tailing, retention time shifts, analyte degradation, etc.) and, therefore, instrument maintenance is very important. Proper cleanup techniques will minimize the problem and instrument QC will indicate when instrument maintenance is necessary.

11.8 Calculations and data handling: Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

11.9 The concentration of each analyte in the sample may be determined by calculating the amount of standard injected, from the peak response, using the mean CF or RF from the initial calibration, or another appropriate calibration model.

12.0 Data Analysis and Calculations

See Sec. 11.0 for information on data analysis and calibration.

13.0 Method Performance

13.1 Performance data and related information are provided only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on an assay specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When waste cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult Less is Better: Laboratory Chemical Management for Waste Reduction available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 Waste Management

WSDA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult The Waste Management Manual for Laboratory Personnel available from the American Chemical Society.

16.0 References

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17.0 Acknowledgements

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